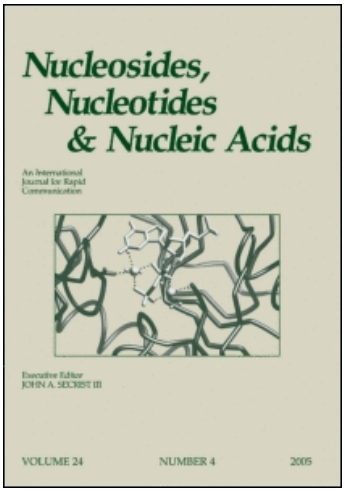


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Di- And Tri-Substituted Adenosine Derivatives and Their Affinities at Human Adenosine Receptor Subtypes

R. Volpini[a]; E. Camaioni[a]; S. Costanzi[a]; S. Vittori[a]; K. -N. Klotz[ab]; G. Cristalli[a]
[a] Dipartimento di Scienze Chimiche, Università di Camerino, Camerino (MC), Italy [b] Institut für Pharmakologie und Toxikologie, Universität of Würzburg, Würzburg, Germany

To cite this Article Volpini, R. , Camaioni, E. , Costanzi, S. , Vittori, S. , Klotz, K. -N. and Cristalli, G.(1999) 'Synthesis of Di- And Tri-Substituted Adenosine Derivatives and Their Affinities at Human Adenosine Receptor Subtypes', Nucleosides, Nucleotides and Nucleic Acids, 18: 11, 2511 — 2520
To link to this Article: DOI: 10.1080/07328319908044623
URL: http://dx.doi.org/10.1080/07328319908044623

NUCLEOSIDES & NUCLEOTIDES, 18(11&12), 2511–2520 (1999)

SYNTHESIS OF DI- AND TRI-SUBSTITUTED ADENOSINE DERIVATIVES AND THEIR AFFINITIES AT HUMAN ADENOSINE RECEPTOR SUBTYPES.

R. Volpini, E. Camaioni, S. Costanzi, S. Vittori, K.-N. Klotz,[a] G. Cristalli.*

Dipartimento di Scienze Chimiche, Università di Camerino, I-62032 Camerino (MC), Italy
[a]Institut für Pharmakologie und Toxikologie, Universität of Würzburg, D-97078 Würzburg, Germany.

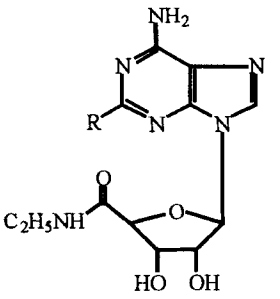
ABSTRACT: The synthesis of 2-(hex-1-ynyl)adenosine derivatives substituted at the N^6- and/or 5'-position was carried out on the basis that 2-(hex-1-ynyl)adenosine-5'-N-ethyluronamide (HENECA, **2**) showed good affinity and different degree of selectivity for rat adenosine receptors. All new compounds were tested in radioligand binding and adenylyl cyclase assays with recently cloned human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors.

Introduction

Adenosine is known to modulate a number of physiological functions through the activation of four receptor subtypes denominated: A_1, A_{2A}, A_{2B} and A_3.[1] Over the last few years much effort has been directed towards the discovery of potent and selective adenosine agonists.

Recently, we have reported on the synthesis of a series of 2-alkynyl and 2-aralkynyl derivatives of 9-(*N*-ethyl-β-D-ribofuranuronamide)adenine (NECA, **1**, Figure 1), which possess high affinity towards rat A_{2A} receptor and good A_{2A} *vs* A_1 selectivity.[2-4]

The finding that the 9-(*N*-ethyl-ß-D-ribofuranuronamide)-2-(hex-1-ynyl)adenine (HENECA, **2**, Figure 1)[5] and some other 2-alkynyl derivatives of NECA[6] also showed good affinity for A_3 receptor subtype prompted us to investigate the effect of various substituents at different positions in compound **2**. For this purpose, the ethyl substituent of the carboxamido group in the 5'-position of HENECA (**2**) was replaced with a cyclopentyl or benzyl group (compounds **6** and **7**, Scheme 1).



1; NECA R = H

2; HENECA R = C≡C–$(CH_2)_3CH_3$

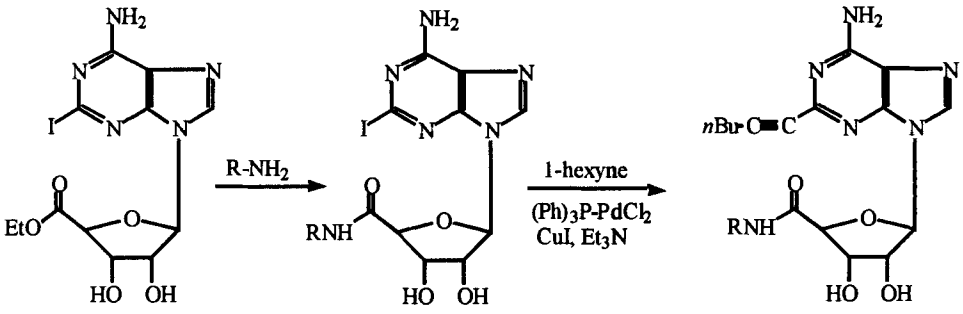
FIGURE 1

A cyclopentyl or arylcarbamoyl group was introduced into the 6-amino group of the adenine moiety (compounds **11**, **12**, and **13**, Table 1) since NECA analogues containing N^6-arylcarbamoyl groups, like N^6-[(3-chlorophenyl)carbamoyl] and N^6-[(4-methoxyphenyl)carbamoyl]adenosine-5'-N-methyluronamides, have shown high affinity towards rat A_1 and A_3 receptor subtypes.[7]

These synthetic goals were achieved by functionalizing the 5'-position with an ethyl ester and positions 2 and 6 with an iodine to obtain the versatile intermediate **9** (Scheme 2). Hence, 5'-(ar)alkylcarboxamido nucleosides were obtained by reacting the corresponding ethyl ester with the appropriate amines, whereas the hex-1-ynyl chain was introduced at the 2-position by a modification of the palladium catalyzed cross-coupling reaction. The 6-substituted nucleoside **11** was synthesized by reacting the corresponding iodo derivative with cyclopentylamine, whereas in the case of ureic compounds **12** and **13** (Table 1) elaboration of the 6-amino group was achieved by reaction with the corresponding isocyanates.

The synthesis and rat receptor affinity of the trisubstituted adenosine derivatives **12** and **13** were reported elsewhere.[8] In this paper, the synthesis of the 2,5'-disubstituted (**6**, 7) and 2,5',N^6-trisubstituted (**11**) adenosine derivatives is described.

All compounds, indicated by the general formula in Table 1, were tested in radioligand binding and adenylyl cyclase assays[9] to assess their affinities for the recently cloned human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors.



3

4 R = cC_5H_9
5 R = $CH_2C_6H_5$

6 R = cC_5H_9
7 R = $CH_2C_6H_5$

SCHEME 1

Chemistry

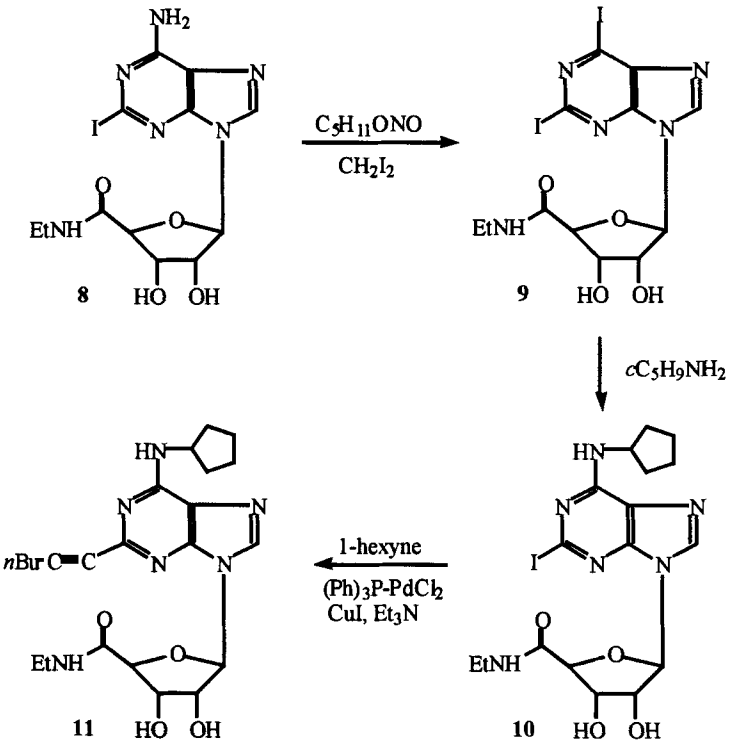
The synthesis of the 2-(hex-1-ynyl)adenosine-5'-N-(ar)alkyluronamides **6** and **7** was accomplished by the reactions described in Scheme 1 using the 2-iodoadenosine-5'-N ethyluronate (**3**) as starting material. Compound **3** was prepared from commercially available guanosine in eight steps.[2] Reaction of **3** with cyclopentylamine or benzylamine, following the general procedure A described in the experimental section, gave the corresponding uronamides **4** and **5**.

Compounds **4** and **5** were in turn reacted with 1-hexyne following a modified palladium catalyzed cross-coupling reaction,[3] as described in the general procedure B, to obtain the N^6,5'-disubstituted adenosine derivatives **6** and **7**.

The trisubstituted adenosine derivative **11** was synthesized starting from the 2-iodoadenosine-5'-N-ethyluronamide (2-iodoNECA, **8**), obtained by treating **3** with ethylamine. [2] The 2-iodoNECA (**8**) was reacted with isopentylnitrite and diiodomethane to yield the 2,6-diiodo derivative **9**, which was substituted at the 6-position with cyclopentylamine to give **10**. Reaction of **10** with 1-hexyne, in the usual way as reported in the general procedure B, afforded **11** in good yield (Scheme 2).

Biological Results and Discussion

All the compounds were evaluated with the human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand bindings



SCHEME 2

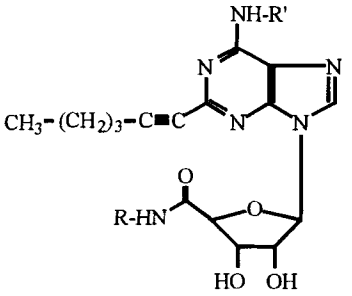
(A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}).[9] Receptor binding affinity was determined using [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) as radioligand for A_1 receptors, whereas [^{3}H]NECA was used for the A_{2A} and A_3 subtypes (Ki; nM).[9] The results are shown in Table 1.

The relative potencies of these compounds for the A_{2B} subtype were measured by evaluating the receptor-stimulated adenylyl cyclase activity expressed as EC_{50}, nM.

The reference compound HENECA (**2**) showed high affinity at A_{2A} and A_3 receptors with a 10-fold and 25-fold selectivity versus A_1 subtype, respectively (Ki A_1 = 60.0 nM, Ki A_{2A} = 6.38 nM, and Ki A_3 = 2.42 nM). The potency for A_{2B} receptor is comparable with that of NECA (**2**: EC_{50} A_{2B} = 6,100 nM *versus* NECA EC_{50} A_{2B} = 2,360 nM).[9]

Replacement of the ethyl group of the 5'-carboxamide function by a cyclopentyl ring brought about an eightfold decrease of affinity at all the receptor subtypes (**6**: Ki A_1 = 403 nM, Ki A_{2A} = 49.4 nM, Ki A_3 = 16.2 nM). When a benzyl group is present in the same

TABLE 1. Affinities of the adenosine derivatives **2**, **6**, **7**, **11**, **12** and **13**, in radioligand binding assays at human A_1, A_{2A} and A_3 adenosine receptors, and in cyclase assays at human A_{2B} adenosine receptor.



Cpd	R	R'	Ki or EC_{50} (nM)			
			Ki (A_1)[a]	Ki (A_{2A})[b]	EC_{50} (A_{2b})[c]	Ki (A_3)[d]
2	Et	H	60.0 50.4-71.5	6.38 3.8-10.6	6,100 3,990-9,300	2.42 2.04-2.88
6	cC_5H_9	H	403 204-796	49.4 29.1-83.8	>100,000	16.2 11.2-23.5
7	CH_2Ph	H	1660 1,210-2,28	720 417-1,240	>100,000	246 127-476
11	Et	cC_5H_9	73.4 38.5-140	178 85.3-370	>100,000	64.7 48.1-87.0
12	Et	3-Cl-Ph-NH-CO	62.4 40.3-96.5	>10,000	>100,000	36.4 22.8-58.3
13	Et	4-OCH_3-Ph-NH-CO	32.8 27.0-39.7	>10,000	>100,000	56.7 41.3-77.9

[a] Displacement of specific [^{3}H]CCPA binding in CHO cells, stably transfected with human recombinant A_1 adenosine receptor, expressed as Ki (nM). [b] Displacement of specific [^{3}H]NECA binding in CHO cells, stably transfected with human recombinant A_{2A} adenosine receptor, expressed as Ki (nM). [c] Measurement of receptor-stimulated adenylyl cyclase activity in CHO cells, stably transfected with human recombinant A_{2B} adenosine receptor, expressed as EC_{50} (nM). [d] Displacement of specific [^{3}H]NECA binding in CHO cells, stably transfected with human recombinant A_3 adenosine receptor, expressed as Ki (nM).

position a more pronounced loss in affinity was found, demonstrating that a bulky substituent is less tolerated at the 5'-position (**7**: Ki A_1 = 1,660 nM, Ki A_{2A} = 720 nM, Ki A_3 = 246 nM).

The introduction of an additional substituent at the N^6-position of HENECA led to compounds with comparable or even slightly enhanced affinity for the A_1 receptor (**11**: Ki A_1 = 73.4 nM, **12**: Ki A_1 = 62.4 nM and **13**: Ki A_1 = 32.8 nM) and decreased affinity for the A_{2A} and A_3 receptor subtypes. In particular the presence of an arylcarbamoyl group caused a dramatic decrease in affinity for the A_{2A} receptors (**12** and **13** : Ki A_{2A} > 10,000 nM). Moreover, all compounds showed no activity at A_{2B} receptors in concentration up to 100,000 nM.

Furthermore, compounds **12** and **13**, which showed strong discrimination against A_{2A} subtype and showed comparable affinity for the A_1 and A_3 receptors (**12**: Ki A_1 = 62.4 nM and Ki A_3 = 36.4 nM; **13**: Ki A_1 = 32.8 nM and Ki A_3 = 56.7 nM), could be useful tools for the simultaneous, selective stimulation of both A_1 and A_3 subtypes.

Experimental

Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with Varian VXR 300 MHz spectrometer; δ in ppm, *J* in Hz. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on Carlo Erba model 1106 analyser and are within ± 0.4% of theoretical values.

A: General procedure for the preparation of 9-(*N*-(ar)alkyl-ß-D-ribofuranuronamide)-2-iodoadenine (4 and 5). To 2-iodoadenosine-5'-N-ethyluronate (**3**)[2] (300 mg, 0.69 mmol) of the appropriate amine (10 mL) was added and the mixture was stirred at r.t. for 16 h. The excess amine was removed *in vacuo* and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give **4** or **5** as amorphous solids.

9-(*N*-cyclopentyl-ß-D-ribofuranuronamide)-2-iodoadenine (4). The reaction of **3** with cyclopentylamine, followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (90:10), gave **4** (264 mg; 81%); ^{1}H NMR (DMSO-d_6): δ 1.33-1.98 (m,

8H, H-cyclopentyl), 4.06 (m, 1H, H-1 cyclopentyl), 4.19 (m, 1H, H-3'), 4.35 (d, 1H, J = 2.4 Hz, H-4'), 4.58 (m, 1H, H-2'), 5.93 (d, 1H, J = 6.4 Hz, H-1'), 7.77 (br s, 2H, NH_2), 8.08 (d, 1H, J = 7.5 Hz, NH), 8.50 (s, 1H, H-8). Anal. calcd. for $C_{15}H_{19}IN_6O_4$ (474.3): C 37.99; H 4.04; N 17.72. Found: C 37.84; H 3.98; N 17.86.

9-(*N*-benzyl-ß-D-ribofuranuronamide)-2-iodoadenine (5). The reaction of **3** with benzylamine; followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (93:7), gave **5** (198 mg; 58%); ^{1}H NMR (DMSO-d_6): δ 4.22 (m, 1H, H-3'), 4.42 (d, 1H, J = 1.5 Hz, H-4'), 4.46 (m, 2H, CH_2-benzyl), 4.59 (m, 1H, H-2'), 5.94 (d, 1H, J = 7.2 Hz, H-1'), 7.28 (m, 5H, Ph), 7.81 (br s, 2H, NH_2), 8.40 (s, 1H, H-8), 8.74 (m, 1H, NH). Anal. calcd. for $C_{17}H_{17}IN_6O_4$ (496.3): C 41.14; H 3.45; N 16.93. Found: C 41.03; H 3.31; N 16.81.

B: General procedure for the preparation of uronamides 6, 7 and 11.

To a solution of the appropriate 2-iodoadenosine-5'-N-(ar)alkyluronamide (**4** or **5**) or N^6-cyclopentyl-2-iodoadenosine-5'-N-ethyluronamide (**10**) (0.58 mmol) in dry acetonitrile (10 mL), dimethylformamide (5 mL), and triethylamine (2.5 mL) under an atmosphere of N_2 was added bis(triphenylphosphine)palladium dichloride (8.1 mg, 0.012 mmol) and cuprous iodide (0.58 mg, 0.003 mmol). 1-Hexyne (2.9 mmol) was added to the mixture, which was stirred at r.t. for 24 h under an atmosphere of N_2. The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give **6**, **7** or **11** as chromatographically pure solids.

9-(*N*-cyclopentyl-β-D-ribofuranuronamide)-2-(hex-1-ynyl)adenine (6). The reaction of **4** with 1-hexyne, followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (92:8), gave **6** (120 mg; 48%), mp 118-120 °C; ^{1}H NMR (DMSO-d_6): δ 0.92 (t, 3H, J = 7.0 Hz, $(CH_2)_3$-CH_3), 1.32-1.97 (m, 12H, H-cyclopentyl and $(CH_2)_2$-CH_3), 2.42 (t, 2H, J = 6.6 Hz, CH_2-C≡C), 4.06 (m, 1H, H-1 cyclopentyl), 4.18 (m, 1H, H-3'), 4.33 (d, 1H, J = 2.0 Hz, H-4'), 4.64 (m, 1H, H-2'), 5.97 (d, 1H, J = 6.5 Hz, H-1'), 7.46 (br s, 2H, NH_2), 8.03 (d, 1H, J = 7.5 Hz, NH), 8.57 (s, 1H, H-8). Anal. calcd. for $C_{21}H_{28}N_6O_4$ (428.5): C 58.86; H 6.59; N 19.61. Found: C 58.73; H 6.47; N 19.73.

9-(*N*-benzyl-β-D-ribofuranuronamide)-2-(hex-1-ynyl)adenine (7). The reaction of **5** with 1-hexyne, followed by chromatography on a silica gel column eluted with $CHCl_3$-

MeOH (90:10), gave **7** (96 mg; 38%), mp 139-142 °C; ^{1}H NMR (DMSO-d_6): δ 0.83 (t, 3H, *J* = 7.0 Hz, $(CH_2)_3$-*CH_3*), 1.33 (m, 4H, *$(CH_2)_2$*-CH_3), 2.18 (t, 2H, *J* = 6.6 Hz, CH_2-C≡C), 4.15 (m, 1H, H-3'), 4.39 (d, 1H, *J* = 1.1 Hz, H-4'), 4.52 (m, 2H, CH_2-Ph), 4.64 (m, 1H, H-2'), 5.98 (d, 1H, *J* = 7.7 Hz, H-1'), 7.28 (m, 5H, H-Ph), 7.60 (br s, 2H, NH_2), 8.43 (s, 1H, H-8), 9.49 (m, 1H, NH). Anal. calcd. for $C_{23}H_{26}N_6O_4$ (450.5): C 61.32; H 5.82; N 18.65. Found: C 61.20; H 5.69; 18.76.

9-(*N*-Ethyl-β-D-ribofuranuronamide)-2,6-diiodoadenine (9). To a solution of **8**[9] (250 mg, 0.58 mmol) in dry DMF (10 mL) isopentyl nitrite (0.86 mL, 6.4 mmol) and diiodomethane (2.82 mL, 35.0 mmol) were added and the mixture was heated at 85 °C for 2 h. The solvent was removed *in vacuo* and the residue flash chromatographed on a silica gel column eluted with $CHCl_3$-MeOH (98:2) to give **9** as a yellow amorphous solid (142 mg; 45%); ^{1}H NMR (DMSO-d_6): δ 1.04 (t, 3H, *J* = 7.3 Hz, CH_2-*CH_3*), 3.19 (m, 2H, *CH_2*-CH_3), 4.28 (m, 1H, H-3'), 4.36 (s, 1H, H-4'), 4.70 (m, 1H, H-2'), 6.03 (d, 1H, *J* = 6.4 Hz, H-1'), 8.15 (m, 1H, NH), 8.98 (s, 1H, H-8). Anal. calcd. for $C_{12}H_{13}I_2N_5O_4$ (545.1): C 26.44; H 2.40; N 12.85. Found: C 26.32; H 2.31; N 12.96.

N^6-Cyclopentyl-9-(*N*-ethyl-β-D-ribofuranuronamide)-2-iodoadenine (10). To compound **9** (120 mg, 0.22 mmol) cyclopentylamine (10 mL) was added and the mixture was stirred at room temperature for 4 h. The excess amine was evaporated *in vacuo* and the residue purified on a silica gel column eluted with $CHCl_3$-MeOH (96:4) to obtain **10** as a white amorphous solid (80 mg; 73%); ^{1}H NMR (DMSO-d_6): δ 1.07 (t, 3H, *J* = 7.3 Hz, CH_2-*CH_3*), 1.48-2.08 (m, 8H, H-cyclopentyl), 3.23 (m, 2H, *CH_2*-CH_3), 4.18 (m, 1H, H-3'), 4.32 (d, 1H, *J* = 1.7 Hz, H-4'), 4.44 (m, 1H, H-1 cyclopentyl), 4.60 (m, 1H, H-2'), 5.92 (d, 1H, *J* = 7.2 Hz, H-1'), 8.16 (m, 1H, *NH*-CH_2), 8.29 (d, 1H, *J* = 7.7 Hz, *NH*-cC_5H_9), 8.41 (s, 1H, H-8). Anal. calcd. for $C_{17}H_{23}IN_6O_4$ (502.3): C 40.65; H 4.62; N 16.73. Found: C 40.51; H 4.50; N 16.86.

N^6-Cyclopentyl-9-(*N*-ethyl-β-D-ribofuranuronamide)-2-(hex-1-ynyl)adenine (11). The reaction of **10** (0.58 mmol) with 1-hexyne, followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (95:5), gave **11** as a white solid (205 mg; 78%), mp 180-183 °C; ^{1}H NMR (DMSO-d_6): δ 0.93 (t, 3H, *J* = 7.1 Hz, $(CH_2)_3$-*CH_3*), 1.08 (t, 3H, *J* = 7.1 Hz, CH_2-*CH_3*), 1.35-2.07 (m, 12H, H-cyclopentyl and *$(CH_2)_2$*-CH_3), 2.44 (t, 2H, *J* = 6.6 Hz, CH_2-C≡C), 3.31 (m, 2H, *CH_2*-CH_3), 4.12 (m, 1H, H-3'), 4.31 (s, 1H, H-4'), 4.60 (m, 2H, H-1 cyclopentyl and H-2'), 5.94 (d, 1H, *J* = 7.7 Hz, H-1'), 8.03

(d, 1H, J = 7.7 Hz, *NH*-cC_5H_9), 8.43 (s, 1H, H-8), 8.76 (m, 1H, *NH*-CH_2). Anal. calcd. for $C_{23}H_{32}N_6O_4$ (456.5): C 60.51; H 7.06; N 18.41. Found: C 60.40; H 7.13; N 18.54.

Biological Studies

Cloning of the human adenosine receptors, stable transfection of cells, cell culture, membrane preparation, radioligand binding, and adenylyl cyclase activity have been fully described elsewhere.[9] Briefly, all human subtypes were stably transfected into Chinese hamster ovary (CHO) cells in order to be able to study their pharmacological profile in an identical cellular background utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}).

Receptor binding affinity was determined using [^{3}H]CCPA as radioligand at A_1 receptors, whereas [^{3}H]NECA was used at A_{2A} and A_3 subtypes. The procedure was performed as described previously.[10] Due to the lack of a suitable radioligand the relative potency of agonists at A_{2B} adenosine receptors was determined in adenylyl cyclase experiments. The procedure was carried out as described previously with minor modifications.[9]

Acknowledgement

Supported by EC Contract No BMH4-98-3474 and by a grant from the University of Camerino.

REFERENCES

1. Ralevic, V.; Burnstock, G. *Pharmacol. Rev.* **1998**, *50*, 413-492.
2. Cristalli, G.; Eleuteri, A.; Vittori, S.; Volpini, R.; Lohse, M. J.; Klotz, K.-N. *J. Med. Chem.* **1992**, *35*, 2363-2368.
3. Cristalli, G.; Volpini, R.; Vittori, S.; Camaioni, E.; Monopoli, A.; Conti, A.; Dionisotti, S.; Zocchi, C.; Ongini, E. *J. Med. Chem.* **1994**, *37*, 1720-1726.
4. Cristalli, G.; Volpini, R.; Vittori, S.; Camaioni, E.; Monopoli, A.; Conti, A.; Dionisotti, S.; Zocchi, C.; Ongini, E. *J. Med. Chem.* **1995**, *38*, 1462-1472.
5. Siddiqui, S. M.; Jacobson, K. A.; Esker, J. L.; Olah, M. E.; Ji, X.; Melman, N.; Tiwari, K. N.; Secrist III, J. A.; Schneller, S. W.; Cristalli, G.; Stiles, G. L.; Johnson, C. R.; IJzerman, A.P. *J. Med. Chem.* **1995**, *38*, 1174-1188.
6. Cristalli, G.; Camaioni, E.; Costanzi, S.; Vittori, S.; Klotz, K.-N. *Drug Dev. Res.* **1998**, *45*, 176-181.
7. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Ji, X.; Olah, M.E.; Stiles, G. L.; Dionisotti, S.; Zocchi, C.; Ongini, E.; Jacobson, K.A. *J. Med. Chem.* **1996**, *39*, 802-806.

8. Baraldi, P. G.; Cacciari, B.; Pineda de Las Infantas, M. J.; Romagnoli, R.; Spalluto, G.; Volpini, R.; Costanzi, S.; Vittori, S.; Cristalli, G.; Melman, N.; Park, K.; Ji, X.; Jacobson, K; A. *J. Med. Chem.* **1998**, *41*, 3174-3185
9. Klotz, K.-N.; Hessling, J.; Hegler J.; Owman, B.; Kull, B.; Fredholm, B. B.; Lohse M. J. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1998**, *357*, 1-9.
10. Lohse, M. J. Klotz, K.-N.; Lindenborn, F. J.; Reddington, M.; Schwabe, U.; Olsson R. A. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *336*, 204-210.

Received : 3 / 22 / 99

Accepted : 6 / 1 / 99